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BULLETIN

OF

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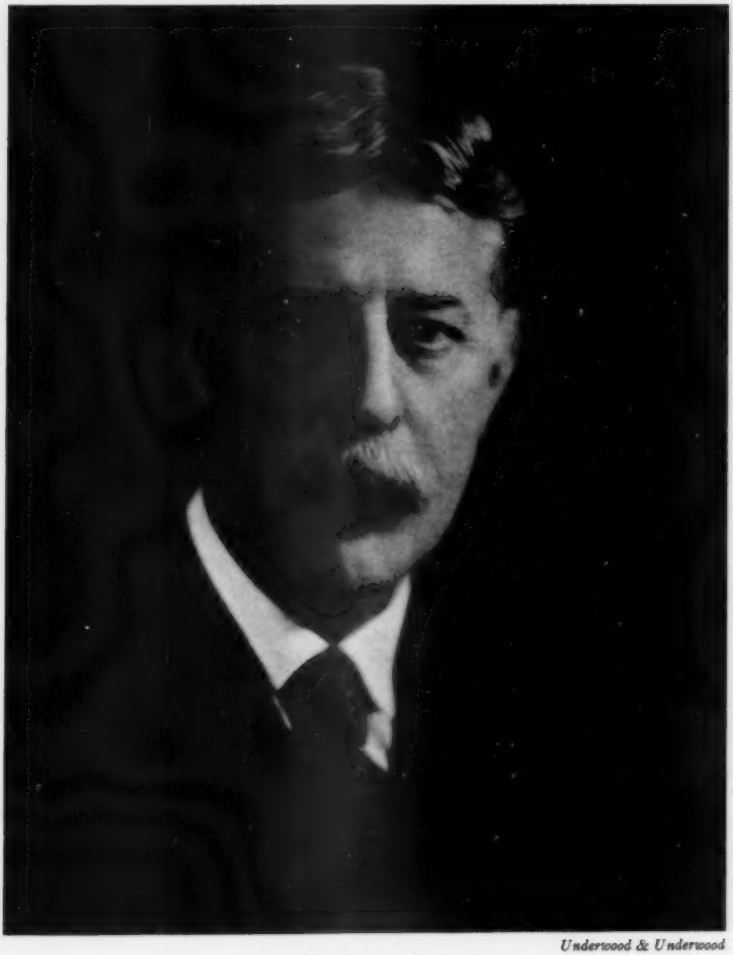

Underwood & Underwood

JOHN WHITRIDGE WILLIAMS

UNIV. OF MICH.

John Whitridge Williams

BORN JANUARY 26, 1866. DIED OCTOBER 21, 1931

A.B. Johns Hopkins University 1886
M.D. University of Maryland 1888
Assistant in Obstetrics, Johns Hopkins University 1889–1893
Associate in Obstetrics, Johns Hopkins University 1893–1896
Associate Professor of Obstetrics, Johns Hopkins University 1896–1899
Professor of Obstetrics, Johns Hopkins University 1899–1931
Obstetrician-in-Chief, Johns Hopkins Hospital 1899–1931
Dean of the Medical Faculty, Johns Hopkins University 1911–1923
Sc.D. (Hon.) University of Maryland 1907
Sc.D. (Hon.) Trinity College, Dublin 1912
LL.D. (Hon.) University of Pittsburgh 1915

"It is with a profound sense of sorrow and of great loss that the Advisory Board of the Medical Faculty of the Johns Hopkins University desires by this minute to record the death of Dr. John Whitridge Williams on Wednesday, October 21st, 1931, and to express its respect and affection for the memory of one who devoted his whole career as teacher and investigator, and for many years as Dean, to the welfare of this Medical School.

"His medical career corresponds in point of time with the whole development of the Johns Hopkins Medical School and Hospital, a development which, as Dean, he did so much to forward. Through all those forty-two years he served not only faithfully and well, but as a tower of strength and inspiration to students and colleagues. Through his own investigations, his remarkable text-book, his organization of the Obstetrical Clinic, his vivid teaching and his relations with his staff and with the obstetricians of this country and of the world, he has influenced profoundly the development and perfection of the science of obstetrics and has set a standard which will be difficult to maintain. His students and assistants occupy many of the most important chairs of obstetrics.

"He was an honored member of many learned societies and several universities in this country and abroad conferred upon him honorary degrees.

"Others will write in detail for the world at large of his services to medicine, and his memory will be enshrined in the respect and gratitude of his colleagues, students and friends."

Extract from the minutes of the Advisory Board of the Medical Faculty, Johns Hopkins University, October 30, 1931.

RECURRENT COMPLETE HEART-BLOCK

Report of a Case Associated With Transient Bundle-branch Block and Normal Conduction Between Attacks[1]

EDWARD P. CARTER, M.D., and DONALD McEACHERN, M.D.

From the Cardiographic Laboratory of the Johns Hopkins Hospital and University

The patient whose illness is here reported illustrated a number of unusual and interesting points with regard to the cardiac mechanism and its reaction to various drugs. Briefly these were:

(1) Recurrent complete auriculo-ventricular dissociation (and Adams-Stokes seizures) with normal conduction between attacks.

(2) Paroxysmal bundle-branch block.

(3) An idioventricular rhythm, during the periods of complete auriculo-ventricular dissociation, which arose sometimes on the left side and sometimes on the right.

(4) The development of ventricular tachycardia following an overdose of epinephrine intravenously.

(5) The onset of status anginosus during thyroid therapy.

CASE REPORT

G. B. U.33820. Male. Colored. Aet. 63. Admitted October 27, 1930. Discharged December 17, 1930.

C. C. Sharp pains in upper abdomen, chest and left side of neck. Attacks of "blind staggers."

F. H. Non-contributory.

P. H. General health good up till present illness. Measles, whooping-cough and chicken-pox in childhood. Pneumonia 29 years ago. Typhoid 21 years ago. No scarlet fever or diphtheria. No rheumatic fever. *Head:* no headaches at any time. *Eyes:* vision good, no change of late; occasional mild diplopia; no inflammatory disease. *Nose:* frequent attacks of epistaxis in last few years, no obstruction, colds infrequent. *Ears:* hearing good, tinnitus for 16 years, no aching or discharge. *Teeth*: uppers badly in need of attention, frequent toothache; all lowers removed two years ago. *Throat:* no tonsillitis or sore throat. *Respiratory:*

[1] Submitted for publication September 1, 1931.

no pain, no dyspnoea, no expectoration until the present illness. *Gastro-intestinal:* for about 30 days there have been gastric symptoms characterized by poor appetite, eructation of gas after eating and epigastric discomfort. No vomiting or haematemesis. Bowels regular. Twelve years ago claimed to have had several dark, tarry stools. No haemorrhoids. *Genito-urinary:* Denies gonorrhoea. Chancre 39 years ago which cleared up rapidly without anti-syphilitic treatment; there were no secondary manifestations noted. For the past year a little difficulty in starting stream, some dribbling and dysuria. *Neuro-muscular:* no nervousness, tremors, paralysis or convulsions. His memory has not altered. *Habits:* Meals regular. Coffee—4 cups daily. Alcohol—2 pints whisky a week, before prohibition; tobacco, one plug daily. Laborer all his life.

P. I. For three years the patient has had recurring attacks of pain behind the sternum. These are sudden in onset, not always related to effort, and sometimes have lasted for as long as a week. The pain is aching in character and he has occasionally been forced to go to bed because of it. Between times he has worked. There has been no dyspnoea and no evidence of myocardial decompensation.

Two years ago he commenced to have attacks of "blind staggers" characterized by great weakness, fading of his vision—often to the point of blindness, great shortness of breath, nervousness and sweating. In the first attack he fell off a wagon and injured his back. His second attack occurred a month later and during it he became blind, felt very weak and almost fell. A severe aching pain appeared in his epigastrium, over the precordium and up into the left side of the neck. It was jumpy in character and like a toothache. He was so short of breath that his tongue hung out and the sweat poured from him. These attacks were sudden in onset, lasted 2 to 3 minutes and passed off suddenly. Two to five attacks occurred each day and he has suffered from them ever since. For two weeks the "misery" in his chest and the side of his neck and head has been constant and he has been very short of breath. During the same time there has been considerable belching and loss of appetite.

P. E. T. 99.6. P. 33. R. 20. A robust, well preserved and well nourished negro of 63. There is moderate dyspnoea when he lies flat. Sclerae pigmented brown. The mucous membranes are of good color. Extra-ocular movements normal. The pupils are equal, round and active to light and accommodation. There is no cyanosis. No sinus or mastoid tenderness. Ears normal. Nose normal. The lower teeth have been removed, the upper stumps are foul and show many cavities. Tonsils are not enlarged nor infected. Pharynx clear. The neck shows no adenopathy and the thyroid is not enlarged. *Chest:* large but symmetrical, and moves freely. Respirations are very irregular and tend to fall synchronously with the pulse which is very slow; they break from its rhythm every few beats, however. The lung bases descend normally and the percussion note is normal throughout. There are no adventitious sounds and there are no râles at the bases. *Cardiovascular:* Blood-pressure 200/80. Eye-grounds: the discs are normal. In the left fundus there is moderate narrowing of the arterial light

streaks and a little cutting at the arterio-venous crossings. The right fundus seems normal. Radial pulses are equal, regular and of high tension; the rate is 32 per minute. The vessels are thickened but not calcified. There is no collapsing quality to the pulse. The heart is greatly enlarged both to the right and left. M.D.L. is 12.5 cm. from the midline in the 5th interspace. M.D.R. is 5.5 cm. from the midline in the 4th interspace. The retromanubrial dulness measures 3.5 cm. to the right and 3.0 cm. to the left in the first interspace. Systolic pulsation is seen in the 4th left interspace near the sternum and also in the 5th interspace in the left anterior axillary line. In the latter area the double character of the pulsation is easily seen and felt as a double thrust during systole. In the same area very small rhythmic pulsations are seen during ventricular diastole; these can be counted at about 70 per minute and are evidently caused by auricular contraction. When they happen to fall with ventricular systole the apical impulse is augmented. In the veins of the neck the same phenomenon can be noted and the waves of auricular origin are easily counted, as they appear to be independent of the ventricular waves. The first sound at the apex is not loud, it is split and is followed by a moderately low-pitched systolic murmur. During diastole faint sounds can be heard falling rhythmically; they are evidently due to auricular systole and when they fall with ventricular systole the first sound at the apex is sharp and much augmented. No diastolic murmur is heard. The sounds at the base are rather faint and not distinctive. *Abdomen:* normal. Liver and spleen not palpable. No masses, no fluid. *Genitalia:* normal. *Lymphatic System:* There is no general glandular enlargement. *Prostate:* of normal consistence, slightly and diffusely enlarged, no nodules; rectal sphincter of good tone. *Extremities:* No oedema, no clubbing of fingers or toes, no roughening of tibiae. *Reflexes:* K. K., A. J. and Plantars are equal and active.

Clinical impression: General and coronary arteriosclerosis. Complete auriculo-ventricular dissociation, with Adams-Stokes attacks. Bundle-branch block.

Laboratory reports: R. B. C. 3,930,000. Hgb. 77 per cent, W. B. C. 9300, Differential count and smear normal.

Blood Wassermann—negative. Urine: SG. 1025, Sugar O. Albumin O. Microscopically negative.

X-Ray of chest: Enlarged heart and aorta; slight fibroid tuberculosis of apices. Teleoroentgenogram: M.R. 5.0 cm. M.L. 11.7 cm. T. 30.0 cm.

Progress notes

On October 29, 1930, during an examination he complained of a "blind stagger" attack. The pulse was felt at once and the beats found to be regular but widely spaced, giving a rate estimated to be about 12 to 15 per minute. The pulse increased in rate, and for the next quarter minute was counted at 24 per minute, during which time the attack passed off and the patient complained of no more symptoms. During the next minute the pulse rose to the usual figure of 29. No muscular twitching occurred. No irregularity of the pulse was noted.

Atropine test: November 2, 1930, 2 mg. of atropine sulphate subcutaneously caused dilatation of the pupils and dryness of the mouth but no satisfactory increase of either auricular or ventricular rate in the electrocardiogram. The following day 2 mg. of atropine were given intravenously, and three minutes later the auricular rate had increased from 53 to 70 per minute, while the ventricular rate showed no change, remaining at 28 per minute. The complete dissociation persisted.

Epinephrine test: November 7, 1930, during a period of complete dissociation and idioventricular rhythm, 0.5 cc. of 1:1000 solution of epinephrine was injected intravenously, through an error. The patient at once complained of great dyspnoea and substernal oppression, and was forced to empty his bladder. The pulse rate rose rapidly. Electrocardiographic records taken after the injection showed numerous extrasystoles arising from various foci in the right and left ventricles, and also from the main bundle. These gave an increasingly rapid ventricular rate. At the end of 30 seconds the auricular rate was 96 and the ventricular rate approximately 60. Two minutes after the injection there was a regular, ventricular tachycardia with a rate of 163 per minute (see figure 6). Four minutes after the injection the idioventricular rhythm had returned, with complexes of the usual type. At seven minutes after the injection the auricular rate was 63 and the ventricular rate 45. The substernal oppression had largely disappeared by this time.

Effect of exercise: March 25, 1930, an electrocardiogram having been taken, the patient was exercised until he became quite dyspnoeic, following which another record was taken. There was no increase either of auricular or ventricular rate, the rates being 77 and 29 per minute, respectively, both before and after the test.

Effect of thyroid feeding: Because of the good results reported in Willius' (7) first paper, thyroid feeding was commenced in the hope of alleviating the Stokes-Adams attacks. Basal metabolism readings were difficult to obtain because of the irregular breathing, but after several trials, and when test periods were run 8 and 10 minutes, satisfactory readings were achieved. Thyroid extract by mouth was commenced on November 11, 1930 in doses of 0.3 gm. t.i.d. There appeared to be no beneficial effect on the attacks, for they continued to occur throughout the treatment once, twice or several times in each twenty-four hours. Distressing symptoms of another nature were, however, observed. For five days following the commencement of thyroid extract no effects were noted; the patient then commenced to complain of shortness of breath, at first on walking about the ward and later at complete rest. He also complained of constant aching pain in the left side of his neck and at times over the precordium and down the inner side of his left arm. The pain was increased when he tried to walk about, and he became "so weak that he had to hold on to the bed-posts" at times. There was no cutaneous hyperaesthesia. His symptoms became more marked, and pain occasionally radiated to the right side of his neck; it interfered with his sleep at night. His appetite disappeared, he declined to leave his bed, his breathing was obviously labored. No change occurred in the physical signs. On November 20th, the end of the ninth day of thyroid feed-

ing, the drug was discontinued. During the next two days the symptoms continued unabated, but on the third day after withdrawal the pain had decreased greatly and he was much more comfortable. His distress rapidly disappeared, and he was soon as active as ever. The basal metabolic rate on November 22nd (the last day of severe symptoms) was plus 49. There was no tremor nor sweating. The basal metabolic rate, taken on numerous occasions subsequently, fell to between −11 and +1.

This is apparently an instance of angina pectoris induced by the added load placed upon the heart through an increased metabolism. A number of similar instances have been described in individuals with normal sinus rhythm, either as a result of hyperthyroidism induced for therapeutic purposes or following the onset of the spontaneous hyperthyroidism of Graves' disease or of adenomatous goitre.

Sturgis (24) has reported cases of myxoedema in which angina pectoris appeared coincidently with the administration of thyroid substance. Christian (10), Means, White and Krantz (23) and Abrami, Brule and Heitz (1) have reported similar instances. Conversely, attacks of angina pectoris in patients with hyperthyroidism have been known to disappear following thyroidectomy (11, 16, 19, 24).

It is of great interest that the pain and distress made its appearance in the present instance despite the fact that the ventricular rate underwent no change. The increased cardiac output per minute in patients with normal sinus rhythm and hyperthyroidism is parallel to the acceleration in heart rate (13). We are not aware of any measurements of the cardiac output per minute in patients with complete heart-block in whom hyperthyroidism has appeared spontaneously or has been induced by thyroid feeding.

It has been shown by Lundsgaard (22), by Liljestrand and Zander (21) and by Alt, Walker and Smith (2) that the resting volume output per minute of the heart in cases of complete heart-block is within normal limits.[2] In view of the slow heart rate in such cases the normal minute flow can be attained only by a great increase in the ventricular

[2] Ellis and Weiss (*Amer. Jour. Med. Sc.*, 1931, **182**, 195) have recently confirmed this fact on four patients with complete heart-block who had no circulatory failure. In view of the low basal metabolic rates noted in their cases it is of interest that in the present case the basal metabolic rate was invariably slightly higher during the periods of complete heart-block than during the periods of normal rhythm (see table 2).

output per stroke. Following exercise, an increase in cardiac outflow per minute occurs in such individuals and is sometimes associated with a parallel increase in ventricular rate. Alt, Walker and Smith (2), however, have made a careful study of one case of complete heart-block in which an increase of cardiac output per minute occurred after exercise, despite the fact that there was no increase in ventricular rate. The increase in cardiac output per minute was achieved in this instance by an enormous increase in the stroke volume output of the heart. Such an increase must of course have been due to an increase in the diastolic filling of the ventricles with a resulting increase in the amount of blood ejected during each systole. It is reasonable to suppose that a similar adjustment may occur in complete heart-block during the presence of hyperthyroidism. No opportunity should be lost in the future to test this point. It is possible that an increased cardiac output per minute may be shown to be present in individuals with hyperthyroidism and complete heart-block, despite the fact that the ventricular rate has undergone no increase. In such an instance the increase in cardiac output per minute would be independent of the ventricular rate. This would suggest that the parallelism that exists between the two, in the presence of normal sinus rhythm, is not necessarily an indication of cause and effect. Studies of the cardiac output per minute in a patient with hyperthyroidism, complete heart-block and auricular fibrillation would be of particular interest, inasmuch as an accelerated rate of auricular contraction could be entirely ruled out as a cause for the increased ventricular filling.

Another question of great interest is the effect of the hyperthyroidism on the rates of auricle and ventricle. There is as yet no adequate explanation of the tachycardia of hyperthyroidism. It has been attributed variously to nervous influences, to direct damage of the myocardium, to simple overwork as a result of the necessity for an increased blood-flow, and to adrenal influences. Experimental work has shown (20) that the hearts and auricles of thyrotoxic animals continue to beat at a greatly enhanced rate after complete isolation from the body. This suggests some change in the metabolism of the muscle. If it can be shown that both auricle and ventricle, in a case of proven complete auriculo-ventricular dissociation, undergo an increase in rate as a result of hyperthyroidism, such would seem to indicate a direct

action of the thyroid substance on cardiac tissue. It would also suggest that the increase in rate is a result of that action. On the other hand, an increase of the auricular rate alone would suggest that the thryoid substance acts directly on the pacemaker or upon the endings of the nerves which control the rate of the pacemaker. An increase in ventricular rate would not be expected under such circumstances on account of the complete block between auricles and ventricles. Epinephrine, for example, is capable of increasing the ventricular rate as well as the auricular rate in cases of complete heart-block in the human being, and in experimental complete heart-block in animals. Epinephrine is known to have a direct metabolic effect on muscular tissue.

In 1918 Aub and Stern (3) reported an interesting case of heart-block in which the auricular rate increased from 75 to 120 under thyroid feeding while the ventricle showed no increase in rate. As the basal metabolic rate fell, after discontinuance of thyroid feeding, the auricular rate fell parallel with it.

Willius in 1924 (26) recanted his previous views (7) and stated that "a careful analysis of a large group of cases of complete heart-block, in which thyroxin or desiccated thyroid had been given, failed to show any appreciable acceleration of ventricular rate. . . . ." He cited one case in which the auricular rate increased from 92 to 180 after 9 days of treatment with thyroxine (0.8 mg. daily by mouth). The ventricular rate remained at 40 to 48 and showed no acceleration. Despite this fact, the Stokes-Adams seizures were benefited and Willius attributed the improvement to the increased output per stroke from the ventricles and the faster blood flow resulting from a dilated arterial bed.

Strauss (25) has reported an interesting instance in which the ventricular rate showed very little or no change either during thyroid feeding or later during spontaneous hyperthyroidism associated with an adenomatous goitre. Following relief of the hyperthyroidism by thyroidectomy, the ventricular rate remained the same.

Drake's case (12) showed an increase of ventricular rate during thyroid medication but it is doubtful if the block was physiologically complete.

A final decision cannot be made on the small number of cases re-

ported to date. The evidence suggests, however, that the idioventricular rhythm in cases of complete heart-block undergoes no increase in rate as a result of hyperthyroidism.

The rates of auricle and ventricle as determined from the electrocardiograms of this patient are tabulated below (table 1). There is certainly no change in ventricular rate. Whether the apparent increase in auricular rate is significant seems doubtful. The rate, previous to thyroid feeding, varied considerably. The figures are presented for what they are worth. It seems strange that exercise

TABLE 1

DATE (1930)	AURICULAR RATE	VENTRICULAR RATE	B.M.R.	REMARKS
October 28	70	21		
November 2	53	29		
November 5	60	27		
November 7	65	33		
November 8				
November 11				Thyroid extract 0.3 gm. t.i.d.
November 15	63	26		
November 17			+35	
November 18	74	28		
November 20				Thyroid extract discontinued
November 21	79	27		
November 22			+49	
November 25	77	29		
November 26	76	27		Reversion to normal rhythm
December 12 and subsequently			−11 to +1	

had no effect on the auricular rate, and that following a large dose of atropine and following large doses of thyroid it never rose above 80 at any time.

REVERSION TO NORMAL RHYTHM WITH NORMAL A-V CONDUCTION TIME

On November 25th the patient had a severe Stokes-Adams attack lasting 3 to 4 minutes, following which the pulse rate was regular at a rate of 70 per minute, and the previous clinical signs of complete heart-block disappeared. An electrocardiogram taken at once showed a

normal sinus rhythm with a P-R interval of 0.20 second (see figure 4). The evidences of intraventricular block remained. During the next few months transition from normal sinus rhythm to complete auriculo-ventricular dissociation, and vice versa, occurred on many occasions, the change usually being associated with a severe attack of "blind staggers." He was equally comfortable with either rhythm, though the breathing was usually irregular during the time of idioventricular rhythm. During the periods of idioventricular rhythm the blood-

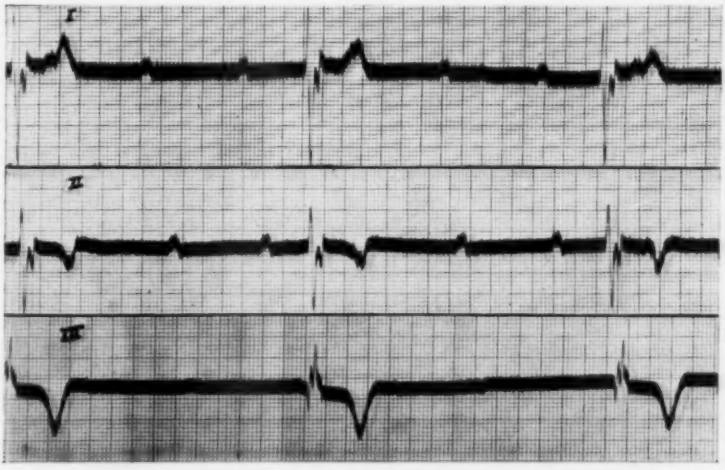

FIG. 1. Complete A-V dissociation. Auricular rate 63. Ventricular rate 20. QRS 0.16 sec. Idioventricular rhythm arising in the right ventricle. The splintering of the QRS complexes and the direction and character of T waves suggest that there is deficient conduction in the left bundle-branch.

pressure was usually about 180/65, and during the periods of normal rhythm about 140/90. These changes are well shown in table 2.

The presence of a normal auriculo-ventricular conduction time in such cases following reversion to normal rhythm is not a common occurrence. Carter and Dieuaide (8) in 1923 were able to collect 8 instances from the literature, and added a case of their own. In the present case the P-R interval varied between 0.18 second and 0.21 second, in electrocardiograms taken during periods of normal rhythm

(see figure 2). During a period of complete auriculo-ventricular dissociation, inhalation of pure oxygen from a mask for 20 minutes had no effect on the block.

TABLE 2

Clinical data

	NORMAL SINUS RHYTHM	COMPLETE HEART BLOCK
Auricular rate	58–65	58–62
Ventricular rate	58–65	25–29
Blood-pressure	155/90	190/70
Vital capacity	49 per cent Normal	42 to 49 per cent Normal
Respiratory rate	18–20 regular	18–24 irregular
Tidal air	380–420 cc.	380–420 cc.
Respiratory quotient	.80	.78
B.M.R.	−10 to −11	−4 to −5

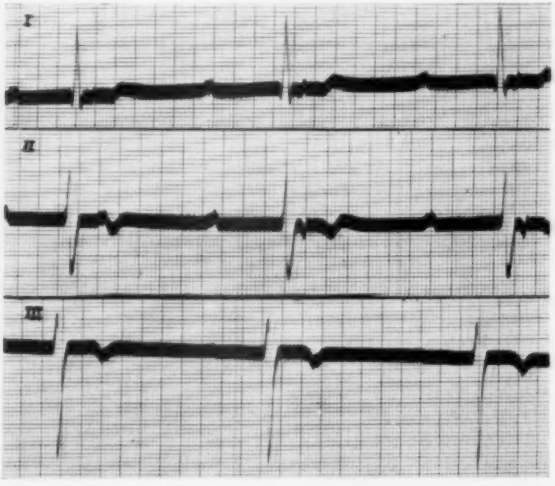

FIG. 2. Complete A-V dissociation. Auricular rate 58. Ventricular rate 28. QRS 0.13 sec. Idioventricular rhythm arising in the left ventricle. There is no evidence of bundle-branch block.

The evidence of Carter and Dieuaide (8) supports the view that the ventricular output per beat may practically double in amount with the

onset of complete block, and that this readjustment may take place abruptly and without conspicuous disturbance of the respiratory rate. Similarly, it is of great interest that in the present case there was such a slight difference between the respiratory rate, the tidal air and the respiratory quotient during the periods of normal sinus rhythm and of complete block. These figures are all extraordinary evidence of the remarkable capacity for readjustment which exists in the cardio-respiratory correlation (see table 2).

PAROXYSMAL BUNDLE-BRANCH BLOCK

The first electrocardiogram taken upon admission showed, in addition to the complete auriculo-ventricular dissociation, the evidences of bundle-branch block (fig. 1). Not only was the rhythm idioventricular but the QRS complexes were heavily splintered, and the T waves were of opposite sign to the main ventricular deflections in all leads. All subsequent records taken during periods of complete heart-block showed no splintering of the ventricular deflections or T waves of opposite sign, although the QRS interval was always longer than normal (figs. 2 and 3). The latter records were thought to indicate that the rhythm was being generated in one or the other branch of the bundle of His without any blockage of spread of the impulse in either bundle. During the periods of normal rhythm, on the other hand, the evidences of right bundle-branch block[3] were constantly present (fig. 4). It was assumed, therefore, that the impairment of conduction in the branch of the bundle of His was not complete, and that, with the prolonged rest allowed by the slow idioventricular rhythm, recovery in the bundle was sufficient to allow transmission of the impulse. A record obtained on April 6th, 1931, showed that such was indeed the case (fig. 5). Normal sinus rhythm and right bundle-branch block were present. Occasionally, however, the ventricle failed to respond to an auricular impulse and a long intersystolic period occurred. The first ventricular complex following this pause invariably failed to show the evidences of bundle-branch block. Inhalation of pure oxygen from a mask for 20

[3] In view of the recent work of F. N. Wilson and his colleagues (6) it should be stated that in this article the old terminology is adhered to, *viz.*: upright QRS in lead I and downward in lead III, in association with the other evidences of bundle-branch block, is considered to indicate involvement of the right bundle.

minutes had no effect on the evidences of bundle-branch block during a period of normal sinus rhythm.

The appearance of the bizarre complexes of bundle-branch block in the presence of the fast rate of the normal sinus rhythm, and their disappearance with the onset of the complete block, seem to us peculiarly significant of the presence of a purely functional basis for their occur-

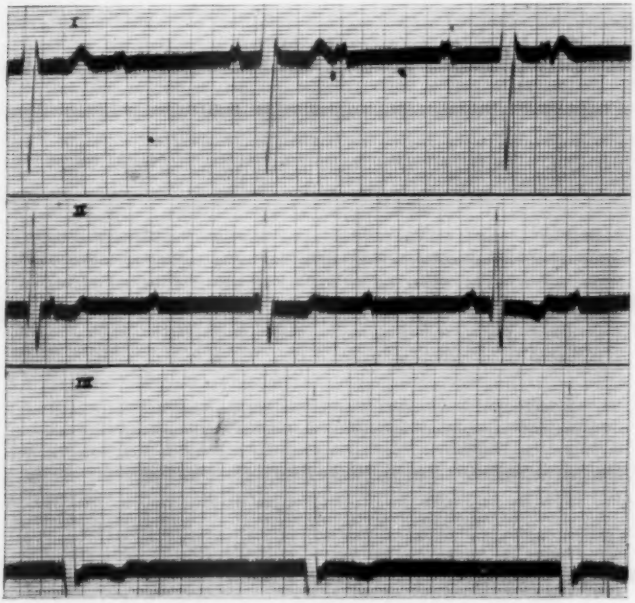

FIG. 3. Complete A-V dissociation. Auricular rate 59. Ventricular rate 27. QRS 0.13 sec. Idioventricular rhythm arising in the right ventricle. There is no evidence of bundle-branch block.

rence, dependent upon an inadequate circulatory supply to the myocardium during the faster rate. We have also thought that it was justifiable to explain the occurrence of the attacks of complete heart block upon an analogous type of functional deficiency, due to inadequate blood supply through the arteria anastomotica magna of Kugel which supplies the A-V node.

Numerous instances have been recorded in which the evidence of bundle-branch block, and even complete auriculo-ventricular dissociation, have been modified, if not altered, by the administration of oxygen or have been induced by a fast rate artificially established. It is of interest that oxygen inhalation did not modify the complete block in the present case. It has also been shown that sclerotic changes, and

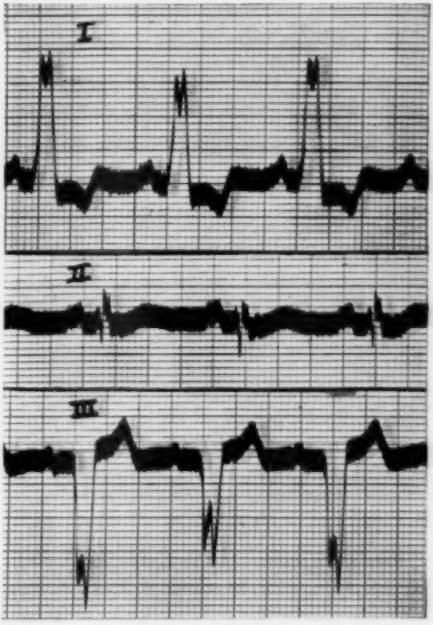

FIG. 4. Normal sinus rhythm. Rate 70. P-R interval 0.20 sec. QRS 0.14 sec. Right bundle-branch block.

partial occlusion by thrombosis of the vessel supplying the A-V node, may be responsible for the occurrence of complete block, and that such changes are of peculiar significance in those cases in which it has been impossible to demonstrate any anatomical injury to the main stem of the His bundle or its branches (14).

An explanation of the occurrence of the widely divergent types of

ventricular complexes and rhythm, that were present in this case, seems possible only upon such an anatomical basis; the result of vascular changes with periods of functional impairment and subsequent

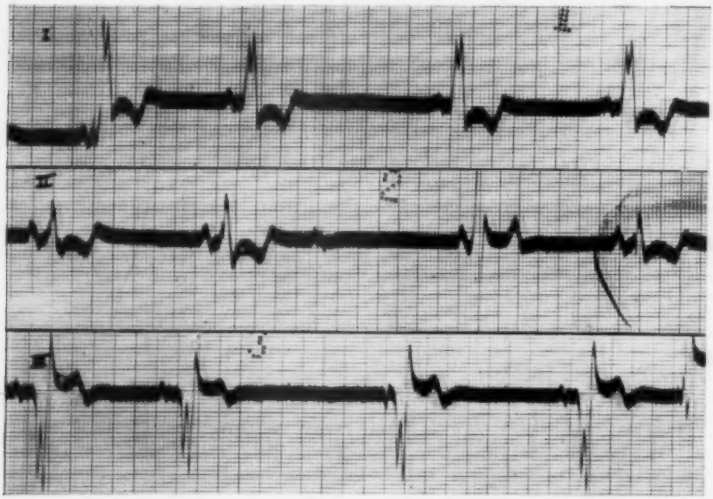

FIG. 5. Normal sinus rhythm, second degree heart block and right bundle-branch block. In lead II the third ventricular complex follows its predecessor after a longer pause (2.46 secs.) than usual. This complex does not exhibit the characters of bundle-branch block.

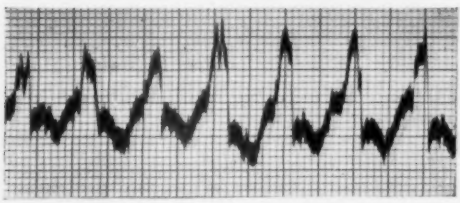

FIG. 6. Ventricular tachycardia following intravenous injection of 0.5 cc. epinephrine. Rate 163.

recovery from the effects of the inadequate blood supply. That the arteria anastomotica magna, or any one of its three branches, may be involved in such cases seems a reasonable conclusion.

VARIABLE ORIGIN OF IDIOVENTRICULAR RHYTHM

Figure 3 illustrates the origin of the idioventricular rhythm in the right ventricle, and figure 2 of its generation in the left ventricle. Numerous records of each type were obtained.

This variation of the idioventricular rhythm was an interesting feature. Utterly independent of any clinical sign or evidence other than the presence of the complete A-V dissociation, the predominant dextrogram or levogram persisted for hours at a time, shifting then from one type to the other, apparently abruptly, to continue until replaced by the preceding type of complex or by the establishment of the normal sinus rhythm. Both the levogram and the dextrogram (figs. 2 and 3) were characterised by an entire absence of the bizarre complexes of bundle-branch block, showing only the increased QRS interval of 0.12 to 0.14 seconds so constantly present in all the records. No record showing an abrupt transition from one to the other type of complex was obtained, that illustrated in figure 1 being the nearest approach to any such transition form. The difference in the end deflection in the latter curves is distinct and significant.

It is of great interest to speculate as to which of the two possible explanations for the occurrence of such shifting rhythms may be the correct one. Are they due to mere transient functional impairment of the bundle-branch involved, or do they represent a true shift of the pacemaker, and the initiation of the ventricular beat at two different sites?

In the majority of the reported instances of such aberrant complexes, they have occurred alternately, or at least the reversion from one type to the other has taken place after a few cycles, and the same type of complex has not persisted for hours as in this case. In the presence of such changing complexes, suggesting either functional impairment or actual shift of the pacemaker, the existence of alternate block of first one bundle-branch and then the other would seem the more obvious mechanism, and yet that such may not be the true explanation is supported by the evidence advanced by Gilchrist and Cohn (15). These writers have reported two such cases in great detail. On the basis of an exact analysis of the ventricular complexes, and a comparison of the time relationships concerned, they conclude that the

mechanism in their cases must depend upon a shift of the pacemaker from one side to the other.

We have not attempted to analyse in this way the records obtained from the case we are reporting. If, however, such a shift of the pacemaker is possible with alternate cycles, it seems to us the most reasonable assumption in the presence of such long continued constancy of the form of the aberrant complex. Furthermore, one record of the idioventricular rhythm was obtained (See fig. 1) which, in addition, showed the characteristics of bundle-branch block.

COMMENT

Quite apart from the unusual combination of disturbances of the cardiac rhythm discussed above, and the appearance of bundle-branch block in the presence of the fast rate with normal conduction, there are a number of interesting clinical points which we desire to emphasize.

We have already referred to the extraordinary facility with which, in the presence of the complete block, the auricular waves could be made out in the jugular vein and over the precordium, and have alluded to the unusual distinctness of the sound of auricular contraction in the long diastole, and the striking change in the loudness or intensity of the first sound at the apex when auricular and ventricular contraction coincided. In addition there was the combination of the auricular and ventricular sounds giving rise to the shifting gallop rhythm heard at the apex either in late or early diastole, just preceding or following the first sound, so often heard as a distinct accompaniment of a complete block.

Of even greater clinical interest, however, was the sharp differentiation which could be made between the visible and palpable auricular waves over the apex in the presence of the complete block, and the reduplicated apex thrust (both visible and palpable) that occurred with the normal rhythm in the presence of bundle-branch block. The clinical signs of bundle-branch block have been described in great detail by King (17) and by King and McEachern (18). To anyone at all familiar with them there should not be any difficulty in postulating correctly from the clinical signs the existence of bundle-block in the large majority of such cases. It is probably true, however, that they might be more readily recognized following an actual demonstration

than from an attempt to convey, by mere description, the peculiar character of the split apical waves in such cases. It is significant that the graphic records in such cases portray the added smaller waves as an event taking place during the chief ventricular contraction (18). Whatever may be the exact physiological explanation, this time relationship to systolic events is a constant and distinguishing feature of these cases. The associated auditory gallop is likewise constant in its time relationship, and is further characterised, in the majority of instances, by the relative feebleness of the sounds at the apex.

Another interesting clinical fact is illustrated by the facility with which the cardiac mechanism shifted from a normal sinus rhythm to a complete heart-block and back again, with no more disturbance than the mild Stokes-Adams syndrome, subjectively described as "blind staggers," and without any conspicuous lengthening of the conduction interval of the normal rhythm. We do not attempt any explanation other than that suggested above, and must regard the phenomenon as being essentially dependent upon the vascular sclerosis. The subject has been discussed in some detail in a previous paper (9).

It does, however, emphasize the critical danger of sudden death that exists with the development of complete heart-block before the age of 50, and the comparative freedom from such early catastrophe in those cases developing after 50 and due so often to cardiovascular sclerosis. It is a matter of record that most of the instances illustrating a normal conduction interval between attacks of complete block have occurred in individuals of advanced age (8). That actual sclerosis of the main stem of the His bundle or its branches may be present in such cases is of course recognized.[4]

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THE TYPICAL POSITION OF MYOCARDIAL SCARS FOLLOWING CORONARY OBSTRUCTION

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It is well known that obstruction of a coronary artery of the heart, slowly produced by arteriosclerosis and often completed by thrombosis, may be survived for a long time. Such obstruction causes the gradual wasting of the cardiac muscle in an area somewhat smaller than that ordinarily supplied by the obstructed artery with replacement by fibrous tissue. In this scarred area the wall of the heart becomes much thinner than it was before and may be stretched out even to form a saccular dilatation of the heart. Thrombi are frequently formed on its inner surface.

It is quite obvious that the area occupied by such a scar must correspond with the distribution of the obstructed artery although, as just stated, it is generally smaller than that because of the anastomoses of the terminals of the coronary arteries now so well recognised. But those who discuss the effects of coronary obstruction seem to disregard the quite characteristic position of the scars which result from closure of right and left coronary arteries. A search through the literature, which is extensive, has ended in our finding only two papers, those of Parkinson and Bedford and of M. B. Whitten, that outline distinctly though briefly the anatomical changes which allow one at a glance to foretell, from the position of the scarred area, which coronary artery is obstructed.

In order once more to direct attention to these self-evident relations, three typical photographs from a long series are herewith reproduced, to show the contrast between the effect of obstruction of the anterior descending branch of the left coronary artery, of its left circumflex branch and of the right coronary artery. These are the common types although, of course, both arteries may be occluded and obstruction of other and smaller branches doubtless produces characteristic scars when this is not obviated by the collateral circulation. An

illustration might have been added to show scarring localised in the anterior papillary muscle in the left ventricle which, as Orth and Amenomiya have shown, is supplied by a special branch from the left coronary.

It will be seen (fig. 1) that obstruction of the anterior descending branch of the left coronary commonly results in scarring of the more

FIG. 1. OBSTRUCTION OF THE ANTERIOR DESCENDING BRANCH OF THE LEFT CORONARY ARTERY

anterior part of the interventricular septum, sometimes even extending through to the right ventricle. and of the apical and more anterior part of the wall of the left ventricle. In sharp contrast with this (fig. 2), the obstruction of the right coronary artery, and especially of the more distal portion which partly encircles the base of the left ventricle and sends branches downward to its wall, produces a scar and dilatation which begins sharply in the mid-line of the interven-

tricular septum and extends backward behind the papillary muscles of the mitral valve to curve round on the more posterior part of the wall of the left ventricle. Scarring of the wall of the right ventricle seems to be rare, and, as Whitten explains, may be due to the more direct course of the branches of this artery which supply it.

FIG. 2. OBSTRUCTION OF RIGHT CORONARY ARTERY

Obstruction of the left circumflex branch of the left coronary artery (fig. 3) results in a scarring and thinning of the wall of the left ventricle in its more median portion, between the province of the left anterior descending branch and that of the right coronary. The interventricular septum is not approached.

Thrombi formed in these sacculations may give rise to emboli which can pass into the branches of the aorta. If the scarring of the interventricular septum is such as to extend through to the endocardium

of the right ventricle, thrombi formed there may give rise to emboli which would pass into the lung.

FIG. 3. OBSTRUCTION OF THE CIRCUMFLEX BRANCH OF THE LEFT CORONARY ARTERY

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THE VITAL STAINING OF RABBIT CARCINOMA[1]

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The earliest and commonest type of experimentation that was carried on for the purpose of studying biologically the true nature of malignant growths was the inoculation of fragments of the tumor into other living animals. Sailer (1) divided these early attempts at transfer into the following groups: inoculation of the lower animals with tumors from other animals or from human beings; inoculation of human beings either with fragments of tumor from some other part of the body or with tumors from other human beings; finally, mediate inoculation with portions of tumors kept for some time outside the body or with cultures obtained from tumors. The number of experiments of this character has been very large. For a review of this phase of the subject the reader is referred to publications by Sailer and by Woglom (2). All efforts to transfer cancer from man to the lower animals have been uniformly failures. Most authorities have agreed that to Hanau (1889) belongs the credit of being the first to transplant carcinoma successfully within the same species, even though his work was preceded by that of two other investigators, Nowinsky (4) (1875) and Wehr (5) (1887), whose experimental reports are frequently omitted from any historical retrospect. That the tumors of Nowinsky and Wehr were infectious granulomata is still open to debate.

THE TRANSFERABLE EPITHELIOMA OF THE RABBIT

Feldman (6) stated that "data are inadequate to justify definite conclusions as to the incidence of neoplasms in the respective species and the predilection of the respective tissues for the various varieties of tumors, but they do demonstrate the fact that neoplasms are not peculiar to any given species but are perhaps ubiquitous for all verte-

[1] Aided by the Garvan Cancer Research Fund.

brates." It is significant also that in an examination of 132 animal tumors, this author (7) found only one tumor (a lymphosarcoma) in the rabbit. Bell and Henrici (8), too, noted that, although the rabbit is extensively used for laboratory purposes and is carefully examined at autopsy, it is rare to find new growths of any kind. Polson (9), in reviewing the literature, gave an estimate, based on the available statistics, that the incidence of tumors among rabbits is 13 out of 1100 animals.

Attempts at transplantation of tumors into rabbits have been numerous. Besides the many experiments of producing cancer in the rabbit by the application of coal-tar products, the attempted transfers have taken two main forms. On the one hand, investigators have used material from human and animal sources for grafting into rabbits, with no success. Duplay and Cazin (10), in 1892, inoculated rabbits, and also rats, guinea-pigs and dogs, by subcutaneous, intraperitoneal and intravenous introduction of fragments of spontaneous carcinoma and sarcoma of the dog, but obtained entirely negative results. Geissler (11), using elaborate aseptic precautions, applied the method of Adamkiewicz (12) of brain transplants in inoculating twenty-five rabbits. The occasional "positive" result was always due to infection; otherwise the animals showed absolutely no symptoms although kept under observation for many months. In those rabbits that were killed for study, the fragments of carcinoma, although fairly well preserved, exhibited no tendency to proliferate, and produced exceedingly little reaction in the surrounding nervous tissue. Paltauf, repeating Adamkiewicz's experiments, obtained negative results. Kopfstein (13) implanted bits of carcinoma into the brains of eight rabbits; and Kinscherf and Bartsch (14) inoculated twelve rabbits' brains, with the identical results of Geissler. Senger (15) introduced human carcinoma either intraperitoneally or intramuscularly into rabbits, mice, and dogs, but invariably failed to produce tumor growth. Paulowski was unsuccessful in his inoculation of frogs, rabbits, and dogs with human carcinomatous material. Ghorghiu (16), using an epithelioma of the mouse, found that gradual resorption of the tumor occurred when grafted subcutaneously into rabbits.

Secondly, iso-transplants in rabbits, spontaneous tumor material being used, have been accomplished with some degree of success. The

work which has been done with the benign rabbit growths—for instance, the myxoma—is familiar to all cancer workers. Sarcomata, however, are the only malignant tumors, with the exception of the epithelioma described by Brown and Pearce, that have been succesfully transplanted into healthy rabbits. No considerable amount of experimental work has been carried out on these animals because, with the exception of the sarcoma of Kato, the tumors have been lost after a few generations of transplantation. Schultze (17), in 1913, found a round-cell sarcoma of the lower jaw of a rabbit with metastatic growths in the liver and the spleen. Six hours after the death of the animal this author and Happe injected an emulsion of the tumor beneath the skin in one series of rabbits and into the anterior chamber of the eye in a second series of rabbits. These transplants resulted in about 80 per cent of growths by the first method and 100 per cent with the intra-ocular injection. The tumor was successfully carried through twelve generations, after which it became necrotic and gradually disappeared. Kato (18) has extended over forty generations the transplantation of a spindle-cell sarcoma which he discovered in the subcutaneous tissue of the back of a rabbit in his laboratory at the Pathological Institute of the Kyoto Imperial University. Gyotaku (19), using Kato's sarcoma, reported successful transplants by inoculation into the eye of rabbits in 94 per cent of the cases. Wallner (20), in 1921, transplanted by various methods fragments from the liver of a rabbit dying with what he supposed to be generalized carcinoma. Histologically, however, the tumor proved to be a mixed-cell sarcoma. Of Wallner's many inoculations the only successful transplant occurred in a rabbit receiving an intravenous injection. This author was unable to carry the tumor beyond a second generation.

Brown and Pearce (21) have published very exact and detailed descriptions of a transplantable epithelial tumor of the rabbit. The tumor arose spontaneously at the site of a primary syphilitic lesion in a rabbit infected four years previously with Treponema pallidum. The appearance of a small, translucent nodule in the left scrotum, corresponding in position to the primary syphilitic lesion, and the growth changes which it exhibited led to its excision for examination. Microscopically, the fact was established that the nodule was of a neoplastic nature rather than a syphilitic granuloma; attempts to demonstrate spirochetes by the darkfield method were unsuccessful.

The early diagnosis of a malignant growth permitted transplantation before the animal succumbed. Success with the transplantation may be attributed to an exact knowledge of the requirements for the growth of a tumor fragment. Brown and Pearce took advantage of the possibilities offered by the testis in being a structure relatively vascular and therefore furnishing an abundantly nutritive field and yet practically devoid of the connective-tissue elements which might participate in the undesirable reaction exhibited by the skin. The correctness of their assumption was evidenced by the high percentage of successful transplantations resulting from intra-testicular inoculations. Treatment with arsphenamine was instituted as a means of eliminating any possible syphilitic infection that might have been transmitted along with the tumor.

Pathologically and clinically the tumor exhibited a high degree of malignancy. The growth, an instance of the development of a malignant tumor upon the basis of a chronic inflammatory lesion, began apparently as an atypical epithelial proliferation. One is forced to recognize, as Brown and Pearce have stated, the difficulty in properly classifying the tumor. The cell type, although it approaches very closely the cells composing the hair follicles of normal epidermis, does not conform exactly to the human squamous or basal cell carcinoma, but may be included under the basal cell variety, and be called an epithelioma. The carcinomatous growths exhibit surprisingly little necrosis, which is found only after the tumor has reached a very large size. As a rule the disease terminates fatally, with death occurring usually from seven weeks to three months after inoculation. The degree of metastasis is variable, but is frequently quite extensive, as these authors have shown in their exhaustive reports.

Through the courtesy of Doctors Brown and Pearce we were furnished with a tumor-bearing animal from which many generations of rabbits have been subsequently inoculated.

THE RETICULO-ENDOTHELIAL SYSTEM AND CANCER

In no other field, perhaps, does more divergence of views abound as to the rôle of the reticulo-endothelial system than in its relation to neoplastic diseases. This system, according to Sacks (22), embraces the reticular and endothelial cells of the spleen, the liver, and the bone

marrow, the lymph nodes, the adrenal and the hypophyseal capillaries, and the entire group of mononuclear phagocytes (histiocytes, macrophages, large wandering mononuclear cells, clasmatocytes, monocytes) of the tissues and the circulating blood. It would be futile and far beyond the scope of this paper to review all the conflicting results reported by cancer research workers; yet it appears advisable to cite some instances to show the utter lack of accord that exists and to give a background for the comparison of the results obtained in our own experimental work.

Weil (23), in 1916, based his conclusion that no definite therapeutic effect could be determined after the use of over twenty dyes of the benzidine group, analogous to congo red, on injection of these compounds into tumor-bearing rats. The author claimed that in the use of these colloidal dyes the dye failed to penetrate living tumor cells, but that the necrotic areas of the tumors presented an intense coloration which he believed depended on a variety of conditions; for example, the degree of diffusibility through the animal membrane, the electrical charge of the dye, its chemical reaction, and its chemical composition.

Karczag, Teschler, and Barok (24) also observed that the necrotic parts of mouse carcinoma adsorb electively such dyes as Fuchsin S, Lichtgrün, and Wasserblau, and explained this attraction of dyes as due to the electrostatic properties of the necrotic area. Their work was seriously questioned by Engle (25), who maintained that only a *living* tumor cell will take up dye. Engle, who used in his experiments grafted carcinoma and sarcoma of the mouse, induced tar cancer of the mouse and rat, and stated that the necrotizing tumor either did not take up the dye stuff or was more lightly colored than the organs. One cannot overlook the fact, however, that all these authors used readily diffusible dyes; and the question naturally arises whether the cells actually stained and lost the dye in the process of fixation, or whether the dye failed to penetrate them in the first instance.

Lignac and Kreuzwendedich von dem Borne (26) believed from their experiments in the vital staining of sarcoma of the white mouse, using trypan blue, that the dye exerted a definitely stimulating influence on the growth of the tumor. They were of the opinion that transplants grew best in dye-stored animals, that there was a greater

number of successful inoculations, that the latent period was shorter, the size of the tumor was greater, and the involvement of the adjoining tissue more extensive, and that there were increased metastases and a shorter length of life of the animal. Moreover, if a tumor growing in a dye-stored animal was grafted back to an animal which had not received any dye—that is to say, an unstored animal—there were seldom, if ever, any successful grafts, while inoculation into other dye-stored animals was accomplished with ease.

Munck (27), on the other hand, did not confirm these observations. He found in mice that the vital storage of trypan blue, with injections begun with or soon after tumor implantation, using both sarcoma and carcinoma, and continued at regular intervals throughout the life of the animal, was not accompanied by a stimulation of growth, but rather that the dye exercised a restraining influence upon the measured circumference of the tumor.

Brüda (28) was inclined to agree with Lignac and Kreuzwendedich von dem Borne in finding animals injected with trypan blue, carmine, and isamin blue more susceptible to cancer transplantation. Brüda reported that in one series which were heavily treated with the dyes the grafts took well, while in another series receiving only a stimulating dose of the same preparation no growths were obtained. This author's failure to give more of his experimental data, especially the number of animals used, leaves room to question his results. Sternberg of Vienna, with his coworkers, v. Eiselsberg and later Psaromitas, stated that at his laboratory they were unable to confirm Brüda's results and failed in grafting Rous sarcoma and human tumor material into mice vitally stained with trypan blue, ink, or iron.

Büngeler (29), in working with ink-injected mice, concluded that it was impossible to transplant a mouse carcinoma after passing it through a Berkefeld filter, whether the material was injected into an animal that had not had an ink injection or into one having received such treatment. He found also that mechanical mincing and treatment with chloroform water so damaged the carcinomatous tissue that it was not possible to produce new growth in a normal mouse by inoculating it with this material because such tissue was always resorbed. Through intensive ink-storage in the reticulo-endothelial system, however, Büngeler believed the defensive forces of the animal

were so blocked that even severely damaged tissue cells could establish growth in the reactionless tissue of the host.

Marsh and Simpson (30) found no specific therapeutic value in administering over 145 dyes derived from coal-tar to mice bearing spontaneous mammary carcinoma. It is not necessary in this paper to give the details of each chemical compound used in their investigation; it will suffice to mention that these workers stated that with subcutaneous injections of trypan blue the tumors were "deeply blued." In the absence of further notes and illustrations one must regard this as a gross observation, and must, therefore, not decide whether the tumor cells were vitally stained or the dye was simply stored in the wandering cells of the stroma.

Ludford's experiments (31) in the various transplantable carcinomata and sarcomata of the mouse tended to show that most tumor cells failed to stain with trypan blue and that the dye had no appreciable effect on the growth of either transplantable or spontaneous mouse tumors. Occasionally, however, he found marginal sarcoma cells with faintly stained granules. Ludford studied minutely the staining of necrotic areas, the stroma, and the wandering tissue cells, and came to the conclusion that the necrotic portions of transplantable sarcomata stained with varying degrees of intensity, that around the tumor areas were deeply stained regions due to the phagocytosis of the colloidal dye by the local fibroblasts and histiocytes, as well as the blood cells, and that the stroma was stained by the dye stuff. It is interesting to note that Ludford observed a considerable variation in the intensity of the macrophage reaction among different types of tumors.

Kato, already referred to above (18), attempted vital staining experiments on his transplantable rabbit sarcoma. This author gave a bare minimum of experimental details and actual observations. He stated that a similar experiment had never been made previously on the rabbit, and that on microscopic examination of the tumor tissue he found very fine carmine granules in the "short spindle tumor cells," and that seldom were particles observed in the "very long spindle-shaped cells of the tumor." The histiocytes, scattered irregularly in the tumor tissues and in the region about the tumor, he claimed, contained "considerably larger and more numerous and distinct granules in their protoplasm." The necrotic portions of the tumor

tissue, Kato remarked, did not show granules, but were even macroscopically revealed to be diffusely stained. It is probable that Kato actually obtained a vital staining of rabbit sarcoma cells; but in view of his meagre descriptions and with a knowledge of the difficulty at times in distinguishing histiocytes and certain tumor cells, his conclusions must remain subject to confirmation.

No more comment is necessary to emphasize further the state of confusion and contradiction which prevails in the literature regarding the vital staining properties of tumors and the relation of the reticuloendothelial system to neoplastic growths. It is evident from the few reports of a previous work just cited that the problem has been approached primarily as a therapeutic one; that is to say, whether a certain dye is growth inhibiting or growth stimulating. Furthermore, except for Kato's work, the rat and the mouse have been the experimental animals employed. Nowhere in the literature were we able to find an instance of the vital staining of rabbit carcinoma.

EXPERIMENTAL WORK

The experimental work may conveniently be divided into two parts, of which the first deals with the injection of one dye only, while the second involves the consecutive injection of two dyes of different colors.

First part. For this series only eight animals were found necessary because the uniformity of the results made a larger series unnecessary. Male rabbits of the Belgian variety about seven months old and weighing from $3\frac{1}{2}$ to $4\frac{3}{4}$ pounds were inoculated into both testes with a saline suspension of the carcinomatous material. The use of both testes doubled the available number of tumors for study. Eleven days after the inoculation the rabbits, bearing visible and palpable tumors, were started on a series of intravenous injections of a one per cent aqueous solution of trypan blue (Gruebler). The injections were made under aseptic conditions, and a one per cent dye solution was freshly prepared for each injection and carefully sterilized before being used. The injections were made as follows.

	cc.
11th day after inoculation	5
12th day after inoculation	10

	cc.
13th day after inoculation	15
14th day after inoculation	15
18th day after inoculation	20
19th day after inoculation	20

The animals were kept under strict observation and were killed as soon as their condition became cachectic. Two rabbits received the first four doses, a total of 45 cc.; five rabbits received five doses or a total of 65 cc.; and one rabbit received six injections with a total of 85 cc. In three instances the rabbits succumbed within three to four hours after an injection; in these cases the autopsy was done immediately. The remaining animals were killed with chloroform, and autopsied at once. Blocks of the tumor of each testis, of the liver, spleen, and kidney, and any metastatic growths were fixed in Bouin's and Zenker's fluid, and in 10 per cent formalin for paraffin sections.

Following the first injection of the dye the animal began to show a blue coloration, particularly of the eye. This coloring increased rapidly with subsequent doses until the skin and indeed the whole animal was intensely blue. Observation of the urine showed extremely little excretion of the trypan blue. At autopsy all the organs were a deep slate-blue color. The liver and the spleen were perhaps more deeply tinted than the other organs. The tumors also were of a deep blue uniform color a trifle more intense than that of the kidneys. The peritoneal fluid seemed to be moderately increased and was of a blue-black color.

The tumor in the gross and on section (Figs. 1 and 2) showed an irregularly lobulated tissue with varying shades of blue in more or less circumscribed areas. Around the periphery of a tiny nodule one could see a well defined rim of deep blue color, while a paler blue center prevailed. The picture of every tumor on section was essentially the same; in some there was a greater amount of cancer invasion with practically complete elimination of testicular tissue; in others the invasion was not so extensive, and the testicular tubules appeared as a compressed mass either centrally or peripherally embedded in cancer. Throughout the section the well defined paler lobules, with occasional scattered deep blue or black dots, and the sharp peripheral outlines of the lobules were striking.

Microscopic sections of the tumors from the different rabbits, and

sections from different portions of the same tumor, were so nearly identical in appearance that a description of one typical section will suffice to give the microscopic picture of the whole group. It was found that sections fixed in Bouin's fluid or formalin demonstrated the dye better than the ones fixed in Zenker's fluid. The sections were counterstained with hematoxylin and eosin. Under low power the trabeculated conformation of the tumor was evident. Strands or cords of a paler staining character tended to circumscribe irregular nodules of deeply staining carcinomatous tissue. Under a higher magnification it was seen that these palely staining strands were a stroma of fibroblasts and cells showing varying stages of degeneration, pyknosis, karyolysis, and karyorrhexis. In such stromatous and necrotic areas one saw an occasional round cell and a number of rather large vesicular cells with small deeply stained nuclei containing definite dark chromatin particles, usually situated toward the periphery of the cells, and with pale pink cytoplasm laden with blue droplets of various amounts. In some cells such a quantity of blue was stored that a coalescence of granules had occurred and the entire cytoplasm presented a lacy blue appearance. By carefully changing focus one was convinced that these cells actually were loaded internally with dye particles and that the blue droplets were not simply adhering (adsorbed) to the surface of the cell. For the most part these blue cells, the monocytes or large wandering mononuclear cells, were to be found in the stromatous trabeculae, but not infrequently they could be seen wandering into the adjacent cancerous tissue. Mononuclear invasion of viable carcinomatous tissue was accidental and irregular, and usually the wandering cells were observed at or near an area of degeneration. Some of the areas undergoing degenerative changes were clearly recognized as compressed testicular tubules, which had been invaded by tumor growth. The fibroblasts had a tendency to leave the stromatous cords, and as offshoots to pierce the adjacent tissue. Some of the fibroblasts contained a small number of the blue dye droplets. Numerous endothelial-lined blood channels were observed throughout, but as far as one was able to determine they bore no peculiar relation to either the stroma or the cancerous tissue except that they seemed to be closely approximated by viable neoplastic cells.

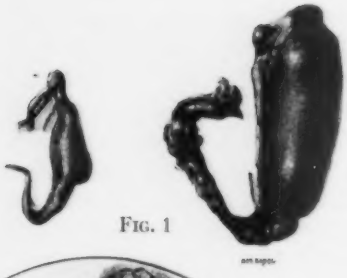

FIG. 1

FIG. 2

FIG. 3

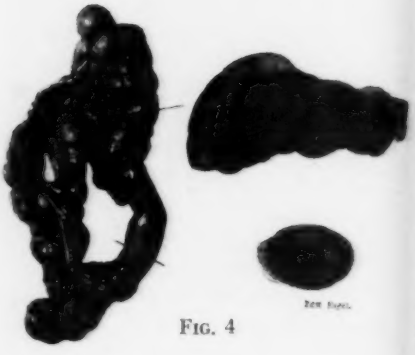

FIG. 4

FIG. 1. A normal testis is seen beside a testis which had been inoculated with carcinomatous material 14 days before its excision. In each case the rabbit had received intravenously a total of 45 cc. of 1 per cent trypan blue (Gruebler). Not only does the testis proper appear eniarged by the neoplastic growth, but a distinct nodule is seen at the region of the globus major and similar nodules are to be seen extending along the cord. The cancer-bearing testis is uniformly colored and of a somewhat darker blue than the normal one. (× 1.)

FIG. 2. A GROSS SECTION OF A TESTIS BEARING RABBIT CARCINOMA, 18 DAYS AFTER INOCULATION

In this case the rabbit had received intravenously a total of 65 cc. of 1 per cent trypan blue (Gruebler). Cancer invasion has been so complete that testicular tissue can no longer be recognized. The entire section presents an irregularly lobulated appearance. Areas of palei staining character are seen to be surrounded by deep blue margins. Scattered throughout are small bluish-black dots

which on microscopic examination proved to be areas of degeneration. Even larger areas of degeneration are seen to be intensely stained with the blue dye. On microscopic examination, however, after the tumor was counterstained with hematoxylin and eosin, the cancerous tissue became darkly stained and the trabeculae and degenerating areas became pink from the eosin.

FIG. 3. A MICROSCOPIC VIEW OF A PORTION OF A CANCER NODULE IN THE TESTIS OF A RABBIT

The section was made from the tumor illustrated in Figure 2. The block of this tumor was fixed in Bouin's fluid, embedded in paraffin, cut to the thickness of 5 micra, and counterstained with hematoxylin and eosin. The drawing was made under oil immersion, a magnification of 1100.

The section shows typical carcinoma cells, in many of which are seen distinct light-blue granules of the trypan blue dye. The cell in the upper left of the field, the cell approximately in the center, and the one slightly to the right of the center show this condition in a marked degree. A comparison of these cells with the other cells in the illustration proves them to be morphologically identifiable as true cancer cells. The cell to the upper right with its cytoplasm laden with light blue dye droplets cannot be definitely identified because in taking the section its nucleus was not present. From its size and shape, however, it is probably a wandering mononuclear cell. This cell represents the type which was so frequently encountered in the areas of degeneration, and serves as an excellent example of the manner in which trypan blue could be contained within a definite cell outline, yet at the same time give a diffuse blue appearance to the area.

The cancer cell in the center shows the trypan blue present in rather discrete large droplets. Close examination reveals the fact that the droplets are contained within the cytoplasm in distinct vacuoles and are not adsorbed to an external cell membrane. The dye particles, which in the illustration appear in the cell nucleus, are actually deeper than the nucleus and are contained within the cytoplasm which surrounds the nucleus. This cell in particular, as well as those cancer cells adjacent to it, shows the remarkable extent to which a carcinoma cell is able to take up a dye, such as trypan blue.

FIG. 4. THE GROSS APPEARANCE OF A 21-DAY-OLD CARCINOMA OF THE TESTIS OF A RABBIT WHICH HAD RECEIVED INTRAVENOUSLY 25 CC. OF 1 PER CENT TRYPAN BLUE FOLLOWED BY 26.5 CC. OF VITAL CARMINE RUBRUM OPTIMUM

Injections of the trypan blue extended over a period of 7 days and the injections of carmine over the 8 days preceding autopsy. The testis proper with its proximal tumor nodules, which have spread by extension, are predominantly stained with the trypan blue, while the distal tumor nodules are of purple color resulting from a mixture of the trypan blue and the carmine. The gross sections on the right show a blue center surrounded by purple lobulated cancer growths. The newer nodules appear to be outlined by the carmine.

In examining a number of areas of unmistakable degeneration and necrosis in various stages, it was evident that no diffuse blue staining of the entire area *per se* had occurred, as reported by many observers. The areas were quite pale and pink; and as a matter of fact, while they did contain a considerable number of blue droplets, it was readily observed that the blue dye was always found to be contained within discrete cells. The intensity of the staining of these cells was in no measure different from that of the large mononuclears seen in the stroma and elsewhere; and from this observation and from the outline of the cell that remained one was justified in identifying these cells as the remnants of wandering mononuclears.

Toward the outer fibrous capsule of the tumor an interesting situation was observed. While this condition was not at all constant and continuous, it was quite apparent in many places that a number of lymphocytes were contained within the capsule, and that between the lymphocytes and the deeply staining cancer tissue were blue, dye-laden, large mononuclears, irregularly situated. In every instance where this circumstance obtained the mononuclears were more numerous than the lymphocytes and were interposed between the lymphocytes and the cancer.

In the circumscribed nodules of neoplastic tissue the uniformity in the appearance of the individual cells was striking. They were fairly compact in arrangement; the individual cells were large and of epithelial type, with a large vesicular nucleus containing distinct, deeply stained chromatin particles and relatively little cytoplasm. The number of mononuclears within the nodule was not great, although occasional ones were seen invading the dense carcinomatous nodule. For the most part the cancer cells appeared free of trypan blue dye droplets. It was not infrequently observed, however, that a cancer cell had actually stored blue dye particles within its cytoplasm. Particularly was this seen near the periphery of the nodule adjacent to the surrounding stroma. Figure 3 illustrates a condition which prevailed in numerous locations within the nodule of tumor tissue. In a clump of tumor cells one could see one or perhaps two such cells, in no way different from the others as far as morphology was concerned, identical in size, character, shape, and position of the nucleus, and containing within the cytoplasm definite blue dye droplets. To

make certain that this was not an adsorption phenomenon with the dye adherent to the exterior of the cell membrane, it was only necessary to change focus and observe that the droplets of dye remained just as distinct as the cell nucleus. Moreover, the fact that a dye-laden cell of identical morphology was directly in the midst of a cluster of unmistakable cancer cells makes the probability of its being anything save a cancer cell extremely unlikely. Furthermore, comparison of a vitally stained cancer cell with a mononuclear wandering cell similarly stained, which in places might be found side by side, readily revealed morphological differences between the two which add to the certainty of differentiation and make positive the assertion that one is really dealing with cancer cells vitally stained with trypan blue.

Microscopic examination of the liver, spleen, and kidney showed a remarkable degree of trypan blue saturation of these organs. The Kuppfer cells and the cells of the reticulo-endothelial system of the spleen were very prominent. Even the liver cells and the cells of the tubules of the kidney showed quite a heavy deposit of blue dye droplets. No pathological changes were noted in the sections of these organs.

Second part. For this series eleven rabbits were used that had been previously inoculated with the rabbit cancer and showed obvious tumor growth. These animals were given intravenously a one per cent aqueous solution of trypan blue and then a suspension of carmine according to the table below. The carmine was prepared as follows:

To a cold saturated solution of lithium carbonate, sufficient Carmine rubrum optimum (Gruebler) was added to make a 5 per cent solution of the dye. The mixture was heated on a water-bath for 15 minutes and filtered just before being used.

The dosages of the two dyes were as shown in Table I.

All of the animals used in this experiment received the same amount of the trypan blue, namely 25 cc., but not all were given the same amount of carmine, which was more toxic than the trypan blue and made it necessary to kill some of the rabbits as soon as their condition became precarious. Four of the series received 11.5 cc. of the carmine, two received 16.5 cc., one received 21.5 cc., and the remaining four received 26.5 cc. of the carmine. Table II gives the total dosages of the two dyes.

The animals were weighed at the beginning of the experiment and at intervals throughout its course. The weight changes may be disregarded since there was such slight variation as to render them insignificant. In general, however, a loss of three to four ounces was observed on the few days immediately preceding death.

Some rabbits succumbed within a few hours after an injection, and in these instances a post-mortem examination was made immediately. The remaining animals were killed with chloroform and autopsied 24 hours after having received their last injection of carmine. Blocks

TABLE I

7th day after inoculation	5	cc. trypan blue (1 per cent)
9th day after inoculation	10	cc. trypan blue
14th day after inoculation	10	cc. trypan blue
16th day after inoculation	5	cc. Carmine rubrum optimum
17th day after inoculation	6.5	cc. Carmine rubrum optimum
21st day after inoculation	5	cc. Carmine rubrum optimum
22d day after inoculation	5	cc. Carmine rubrum optimum
23d day after inoculation	5	cc. Carmine rubrum optimum

TABLE II

	TOTAL TRYPAN BLUE	TOTAL CARMINE
	cc.	*cc.*
Group I (4 rabbits)	25	11.5
Group II (2 rabbits)	25	16.5
Group III (1 rabbit)	25	21.5
Group IV (4 rabbits)	25	26.5

of the tumor of the testes, the organs, and any metastatic growths were fixed in 10 per cent formalin and Bouin's solution for paraffin sections.

By the time an animal had received 25 cc. of the trypan blue solution it showed an intensely blue coloration, particularly noticeable in the eyes. From the previous series in which intravenous injections of trypan blue were given it was known that 25 cc. were sufficient to produce, without giving pronounced toxic symptoms, a deep blue color in all the tissues capable of taking up colloidal material. Moreover, the fact that this dye is excreted very slowly allows of a maintenance of a blue color for about two to three weeks. It was for these

reasons and because the carmine is more rapidly excreted that the trypan blue was administered first. After the injections of the carmine were commenced there was a slight but not a marked change to be noted in the external appearance of the animal. A rather dark blue color of the skin persisted, while the eyes showed a tendency to assume more of a purple hue with each successive injection of the carmine.

At autopsy the organs in the gross showed various degrees of staining. The liver and the spleen were perhaps the most deeply stained. These viscera were of a rather uniform purple tint, the intensity of the purple being dependent upon the amount of carmine which the animal had received. In the animals of Group I the liver and spleen presented an almost decided blue, while these organs in the rabbits of Group IV were a very reddish purple. The kidneys stained variably in the different rabbits. In those animals which received a small amount of the carmine the kidney capsule and cortex on gross section appeared to be a blue color, the calices and pelves of the same kidneys being of a reddish purple. In the animals, however, which received a larger amount of carmine the kidneys were more uniform in color, a reddish purple; their capsule was darker and predominantly a bluish purple and their striae were quite prominent as radial blue, almost black, lines against a reddish purple cortex and pelvis.

The cancers of the rabbits which received a double injection of the two dyes showed no stereotyped picture that could be considered typical of all, yet there were several features which were common to all. In the gross none showed a solid and entirely purple coloration as one might expect to result from an admixture of dyes of blue and red color. Instead, all were mixed colors with separate and distinct blue and purple areas diffusely mingled in irregular patterns in the various tumors. Moreover, comparing the tumors of the various groups—for instance, Group I in which the carmine injection was only 11.5 cc. and Group IV in which the carmine reached a total of 26.5 cc.—it was immediately apparent that the manner in which the tumor stained depended primarily upon the manner in which the tumor had been inoculated, on the way it had grown subsequently, and on its configuration, and only secondarily did its staining reaction depend upon the absolute dosage of the carmine. In other words,

in all the tumors there were blue areas which remained pure blue regardless of the group to which they belonged, and purple areas which tended to become increasingly more of a reddish purple as the amount of the carmine injected increased.

At this point it may be well to call attention to two general types of configuration of the tumor, based perhaps on the method of implantation. Type I may be described as tumor enclosed almost entirely within the capsule of the testis, namely, an intra-testicular growth, which has grown by infiltration, distending this capsule and thus increasing the diameter of the testis. The character of the growth of Type I apparently had resulted from a central inoculation of the cancer. In Type II the original tumor inoculation must have occurred at the periphery of the testis and the inoculating needle pierced the capsule at several points, spreading carcinoma cells on the capsular surface. Type II, therefore, instead of being a compact, regular growth, tended to grow mainly outside the testis in various-sized nodules adherent to the testis and to spread along the cord and mesorchium, with perhaps a smaller proportion of the growth within the testicular substance. In Type II, then, one may see an almost intact and normal looking testis surrounded by dense extra-testicular cancer, or one may find only portions of the testicular tubules invaded by cancer which has spread for the most part on to the capsule and thence along the cord. Tumors of the two types are found in equal numbers in the four groups previously mentioned.

In all of the tumors, whether confined largely to the testis—that is to say, Type I—or whether of the morula-like appearance—that is, Type II—there was a decided tendency on the part of the neoplasm to preserve a lobulated type of growth. On gross section of a tumor of Type I the lobules exhibited paler centers of tumor tissue outlined by more deeply staining margins. In Type II the same method of growth prevailed; that is, the nodules that grew extra-testicularly could be subdivided again into smaller lobules with paler centers and deeper staining edges. Moreover, the staining reaction was not one of simply outlining the small lobules, but within the paler centers of the lobules masses of stained cells were scattered irregularly.

In all four of the groups (based on the amount of carmine received) blue and purple lobules were always found. By comparing the tumors

of Group I, which received 11.5 cc. of carmine, with those of Group IV, which received 26.5 cc. over a longer period of time, it was found (1) that practically no difference existed in the intensity of the blue in the solidly blue lobules of the two groups, (2) that the purple was deeper and more of a bluish color in the tumors of Group I and more of a reddish purple in Group IV, (3) that the tumors of Group I were larger and on the whole more of a blue than the tumors of Group IV, and (4) that in both groups the small adherent nodules (described as characteristic of Type II) were solidly purple and the shade of the purple became more red as the amount of the carmine injected had increased. Thus it seemed that the more remote the growth was from the site of the original implantation, and certainly the younger the growth was, the more solidly reddish purple the lobules appeared to be. Also, the nearer to the site of the original inoculation of the cancer, that is to say, the nearer to the older growths, the more solidly blue the lobule appeared without variation in the intensity of the blue, regardless of the time elapsing after the injections of trypan blue or the amount of carmine subsequently administered.

To demonstrate microscopically the presence of the two dyes within the cells the sections, cut to the thickness of 5 micra, were stained with picric acid, which gave an excellent contrast to the blue and red colors. No widespread diffusion of either the trypan blue or the carmine had occurred. It was interesting to note that the dye appeared as discrete granules of either blue or red, and in no instance was a mixture observed which would have resulted in a purple granule. The purple color noted on inspection of the gross material was caused by the close proximity of numerous vacuoles containing either blue or red dye granules. The phagocytic cells might therefore be divided into three classes,—those containing only trypan blue, those containing only carmine granules, and others with discrete trypan blue in some of the vacuoles and pure red granules in other vacuoles within the cell.

As far as the actual staining of the carcinoma cells themselves was concerned, nothing further can be added to the report already made in the first part of this paper. It was frequently observed that the cancer cells had been vitally stained with either the trypan blue or the carmine, but no instance was found in which a carcinoma cell contained granules of both dyes.

The vitally stained phagocytic cells were not limited to the peripheral areolar tissue, but were present in varying numbers in the stroma which separated the lobules of tumor tissue, and not infrequently by being scattered within the tumor proper, these cells accounted for the irregularly scattered dots of blue and red seen in the gross. Other cells, such as plasma cells, and lymphocytes, also were present in the areolar structure, but their numbers were small compared to the mononuclears.

The phagocytic mononuclear cells arranged themselves about and within the lobules of the cancer in several ways. About one lobule these mononuclears were predominantly, and in some instances entirely, blue. About a second lobule, usually at a little distance from a blue one, red cells were in the majority. Intermediate between the two lobules the mononuclear cells contained a mixture of red and blue vacuoles. Within a lobule of carcinoma cells areas were observed occasionally which, because of pyknosis and karyorrhexis, were suggestive of tissue death. About such areas phagocytic mononuclear cells containing carmine were invariably found, and frequently in large numbers. In these regions it was extremely rare to find cells which contained trypan blue.

DISCUSSION

The application of vital staining technique to cancer investigation, although carried on extensively for many years, has apparently never before been applied to the carcinoma of the rabbit. In considering the observations outlined above, three issues present themselves for discussion; first, the relation of those cells of the reticulo-endothelial system, variously called monocytes, macrophages, large wandering mononuclears, histiocytes, or clasmatocytes, to cancerous tissue; second, the actual vital staining of living carcinoma cells with trypan blue; and third, the double vital staining with trypan blue and carmine.

Descriptions and illustrations have been given to demonstrate the fact that deeply stained areas exist about the cancer tissue (Fig. 2). Such areas result from the vital staining of the large wandering mononuclear cells, whose increase in the circulating blood of the rabbit after carcinoma inoculation has been shown by Pearce and Casey (32). In view of the fact that no inflammation was observed within the

tumor and that large accumulations of monocytes occurred about the tumor, it is possible to substantiate the reports of Goldmann (33), who drew attention to the accumulation of macrophages in areas of tumor inoculation. Ludford (31) also observed in rats and mice a variation in the amount of the deeply stained tissue around the different types of tumor. Furthermore, the relatively small number of lymphocytes present, which Murphy (34) considered the defensive mechanism against cancer invasion, as well as the arrangement of the lymphocytes present—that is to say, the mononuclears were observed adjacent to the carcinoma while the few lymphocytes were external to the monocytic row—and the increase in the number of monocytes, make it seem that the mononuclear cell has an important part in the reaction of the body to the cancer. There is no proof that this rôle is defensive in character, however.

Second, in regard to the actual vital staining of the carcinomatous tissue, the first observation showed that the trypan blue was actually within the cancer cell and was not adsorbed to its exterior. It is striking that in an animal so well saturated with trypan blue, as evidenced by the abundance of the dye present in the organs, the cancer should appear comparatively free of the dye. The cancer cells which showed the vital staining were, for the most part, situated near the periphery of the tumor nodule in rather close approximation to the stromatous trabeculae.

Although many of the cancer cells were vitally stained, nevertheless this total number was only a small fraction of all the cancer cells. However, the possibility still exists that a great many more cancer cells might contain the dye, and the only reason these cells were not identified was that no refined methods for the identification of minute quantities of the dye were available. Since the cancer cells containing the dye were usually situated at the periphery of a lobule, it may be possible to explain the selective action of the cancer cell on the basis that those situated at the periphery are the youngest and most malignant, and have a metabolic activity quite different from the older cancer cells situated near the center of the lobule. Thus Blotevogel (35) found, with the use of 0.5 per cent trypan blue solution, that a greater storage of dye occurred in mice that were young, the dye being found in such structures in the young as the retina, ciliary process,

choroid, sclera, ciliary body, and iris, whereas in the adult animals only the clasmatocytes effected a storage. This phenomenon of increased deposition of the dye, he supposed, was due to an increased metabolism in the young animal. The inference in the case of the peripheral cancer cells is obvious. The effect of age, then, may be (1) a difference of permeability of the external cell membrane; (2) the young cells may be of different pH [Lewis (36)] as a result of their different metabolism, and therefore, on a physicochemical basis, one might postulate a flocculation of the ultra-microscopic particles of the colloid within the cell cytoplasm; or (3) because of immaturity and different metabolism, the young cancer cell might not be capable of readily eliminating the dye stuff, and the result would be dye accumulation within the cell. But to point out the most obvious fact, a phenomenon of selective staining of the cancer cell has occurred, and it indicates perhaps a functional variation in the life cycle of cancer-cell activity.

Third, as regards the results obtained with the use of the two dyes, they were, with one notable exception, in agreement with the observations recorded by other investigators, Evans and Scott (37), Schulemann (38), Smith and his co-workers (39-41), and Steckelmacher (42). However, none of these authors stained cancer vitally with two dyes, and in this respect the double staining herein reported is not only unique for rabbit carcinoma but also for experimental cancer in general.

FIG. 5. A MICROSCOPIC SECTION OF A RABBIT CARCINOMA TO ILLUSTRATE THE REMARKABLE MANNER IN WHICH THE TUMOR NODULES ARE OUTLINED BY VITALLY STAINED MONONUCLEAR CELLS

The cancer was inoculated 20 days before autopsy of the animal, which during the course of the last 14 days of its life received intravenously 25 cc. of 1 per cent trypan blue, followed by 16.5 cc. of vital carmine rubrum optimum. It is at once apparent that the adjacent lobules are circumscribed by cells which are predominantly blue about the periphery of the larger lobule and largely red about the smaller lobule. This fact is further illustrated on the left by the higher-power (x 1100) picture, which shows the dye as discrete granules, either pure blue or pure red. In no instance is there a mixture of the two dyes to form a purple granule. For the most part the cancer lobules outlined by the blue cells represent the older portions of the neoplasms.

Section was fixed in one per cent formalin and cut to a thickness of 5 micra in paraffin.

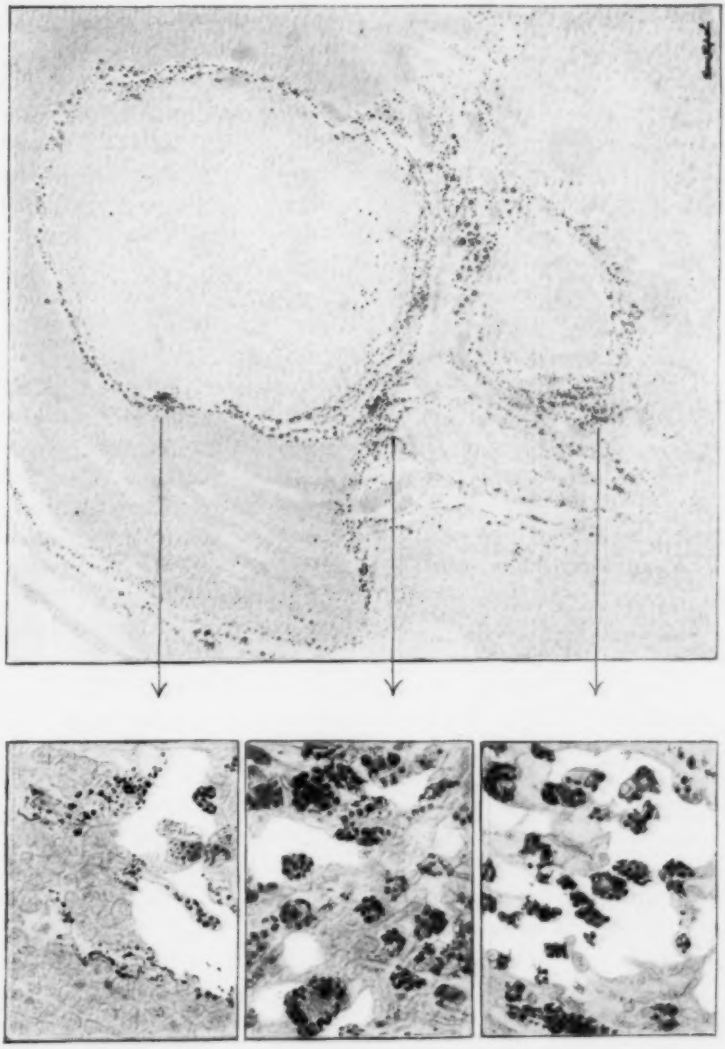

FIG. 5

From the order of administration of the dyes it is reasonable to assume that the cells which were predominantly blue as a result of the storage of the first dye to be injected, that is to say, the trypan blue, were the oldest cells; that the cells containing both blue and red granules were next in age; and that the cells loaded with carmine only were the youngest cells. Since only two days passed between the last administration of the trypan blue and the first carmine injection, it seems impossible to believe that sufficient time had elapsed for a cell to give up all its blue dye and then engulf the carmine secondarily.

These experiments have also brought out a fact which seems to be in contradiction to previous observations. Ordinarily a phagocytic cell cannot be saturated with one dye, for no matter how completely the first dye may seem to have saturated the cell, it may still be able to take up some of the second dye (Smith). However, in this vitally stained carcinoma tissue it was not uncommon to find whole groups of cells filled with trypan blue and containing not a trace of any red dye. Certainly these cells had ample opportunity to ingest the red dye. The obvious explanation would be that phagocytic cells called out by cancer are different physiologically from this class of cells when produced by other agencies.

The use of the two dyes also showed that the vitally stained cells did not wander about very freely, because if such traveling were at all a prominent characteristic of their behavior, it would be reasonable to expect a mixture of cells, some containing only blue granules being near ones having only the red dye. But such a picture was never observed. In fact, one was more impressed with their sessile behavior. The question whether this slowed rate of locomotion was due to the cancer or whether the wandering cells after all travel very slowly must naturally be left open.

The tendency toward the grouping of cells of the same color and the marked contrast in the color of the two dyes employed showed the sequence of events about a growing neoplasm (Figs. 4 and 5). In the loose areolar area about the outside of the cancer and in the stroma about certain of the lobules one found mononuclears containing trypan blue. These lobules were therefore undoubtedly the oldest portions of the cancer. About other lobules were mononuclear cells

containing a mixture of blue and red granules; these lobules were next in age. The lobules circumscribed by mononuclears entirely red in color represented, we believe, the younger cancer growth. In this way, then, one was able to see the direction in which cancer spread had occurred. With the tumors in this experiment there was a constant tendency toward growth upward along the cord. Moreover, many of the tumors had small lobules attached to the outer surface of the testicular capsule. These lobules, in every instance, were outlined by cells having the red dye and represented, even in the absence of any vital staining, isolated areas of new cancer growth.

In many of the lobules there was an extensive mobilization of the mononuclear cells at areas of tissue death as indicated by pyknosis and karyorrhexis. The presence of large numbers of the red stained cells at such areas, the uniformity with which they were seen wherever there was a suggestion of necrosis, and the almost complete absence of cells with the blue dye would seem to indicate that the younger and more vigorous cells would be the first to be attracted to these areas. The fact that the blue stained cells were not seen intermingled with the young red stained cells indicates that the former do not react quickly to the presence of necrotic tissue, and brings further evidence for their relatively sessile character.

SUMMARY

It has been the purpose of this paper to record for the first time the vital staining of rabbit carcinoma by the intravenous administration of different dyes. In the first series of animals, trypan blue alone was injected, and it was found that the dye lodged primarily in the phagocytic wandering mononuclear cells, which were situated in great numbers in the stroma surrounding the tumor nodules. To a much less extent were these cells found within the cancer nodule itself. Only occasionally did the cancer cells take up the dye, and in such instances the cells were situated near the periphery of the nodule.

In the second series of animals, a course of trypan blue was given first, followed by a similar series of injections with carmine. By the use of these two contrasting dyes it was seen that the oldest carcinoma areas stained predominantly blue, the youngest red, while a mixture of these two colors was seen in the region of intermediate cancer

growth. The dye was in the phagocytic cells; some cells contained only the blue, others only the red, the majority containing granules of both dyes; but never were cells seen in which a vacuole contained particles of each dye. The fact that some of these cells had only the blue dye showed that they were incapable of taking up the red. In this respect an instance is brought where a "blockade" of certain cells of the reticulo-endothelial system occurred, and an exception is thus furnished to the general belief that such cells cannot be blocked.

The tremendous number of these cells, particularly in contrast to the relatively few lymphocytes, indicated a peculiar significance for them in cancer growth. Furthermore, their distribution in the cancer area suggested that their ability to move about in the tissues had been exaggerated.

Again, the young or red stained phagocytic cells were always present at the areas of cancer necrosis, even if the surrounding tissue was composed of relatively old cancer cells as judged by the predominantly blue color of the macrophages.

Whether present in the cancer cell itself or in the monocyte, apparently the dyes did not give any indication of retarding cancer growth.

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